EXHIBIT 40

Nucleic Acid Probes

A Primer for Pathologists

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ASCP Press
American Society of Clinical Pathologists
Chicago

Library of Congress Cataloging in Publication Data
Piper, Margaret, 1953–
Nucleic acid probes : a primer for pathologists / Margaret A. Piper, Elizabeth R. Unger.

Includes bibliographical references.
ISBN 0-89189-283-4
1. DNA probes—Diagnostic use—Handbooks, manuals, etc. 2. Nucleic acid probes—Diagnostics use—Handbooks, manuals, etc. I. Unger, Elizabeth R., 1951– . II. Title.
[DNLM: 1. Nucleic Acid Probes. QU 58 P665n]
RB43.8.D63P56 1989
616.07'56—dc20
DNLM/DLC
for Library of Congress. 89-17940
CIP

Printed in the United States of America.

92 91 90 89 4 3 2 1

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hybridized interaction (such as charge or hydrophobic binding)—can greatly compromise the sensitivity and specificity of the assay. The need for rigorous washing and controls in the assay is obvious.

The degree of sample preparation varies greatly among many forms of the assay currently in use. Of course, highly purified DNA or RNA samples may be applied to the matrix. This requires individual handling of each sample through the cell lysis and extraction steps; it also requires a relatively large amount of sample that can be readily extracted. The purified nucleic acid sample is most available for interaction with the probe and is least subject to nonspecific sticking of the probe. The assay is relatively tolerant of degradation of the nucleic acid sample.

While this form of assay was initially used with isolated and relatively pure nucleic acid samples, it has gained popularity because of its ability to test unpurified sample material. If whole cells or crude patient material (such as blood, stool, or sputum) are bound to the solid matrix, a kind of modified DNA extraction procedure may be performed on all of the samples simultaneously. This usually involves a lysis and denaturation step (often using a strong base or a chaotropic agent such as sodium iodide), protein removal (usually an enzymatic digestion and/or sodium dodecyl sulfate treatment), and washing. This forms the most streamlined method of sample handling. While very convenient, this type of assay is much less sensitive than when purified samples are used as the starting material. The difficulty lies in the limited amount of starting material that can be immobilized, as well as in the relative inefficiency of the extraction procedure. In addition, the crude nature of the sample makes the assay much more susceptible to nonspecific binding of probe, yielding high background or nonspecific signals.

Sandwich hybridization

The sandwich hybridization assay (Figure 2–3) is a modification of the dot/blot hybridization assay and was designed to overcome

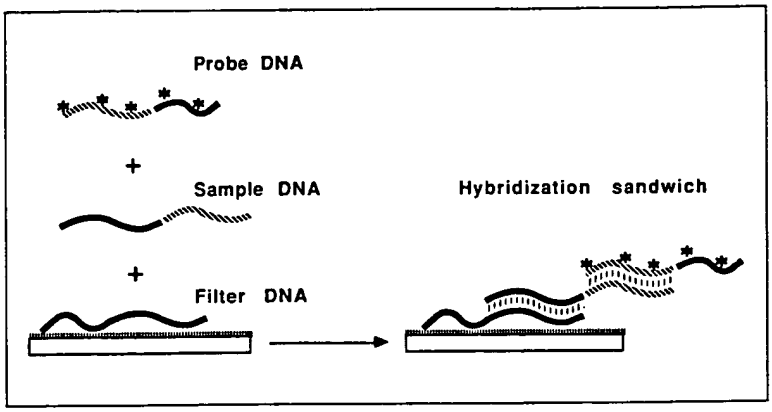

Figure 2–3. Schematic representation of the sandwich hybridization assay.

the problem of nonspecific signal generated with crude samples. The assay requires the purification of two nucleic acid reagents. These sequences are adjacent to one another in the target of interest, but are noncomplementary. One sequence, known as the "filter" or "target" probe, is immobilized on the membrane. The other sequence is the labeled, or detection, probe. The crude sample is interacted with the filter and detection probes simultaneously. Only samples with sequences capable of forming a "sandwich" between the target and probe sequences will generate a signal. This strategy reduces nonspecific signal dramatically. Recently, a related sandwich-type technique termed *affinity-based hybrid collection* was developed (Figure 2–4). Relatively crude sample is interacted in solution phase with target and signal sequences. The difference is that the target sequence is unbound but labeled with an affinity target (such as biotin). Hybrids are collected after the hybridization procedure with the use of the affinity label (such as avidin). Only those samples hybridizing with both target and signal-generating sequences yield a signal. This method combines the advantages of relatively crude sample preparation with the rapid kinetics of solution-phase hybridization.

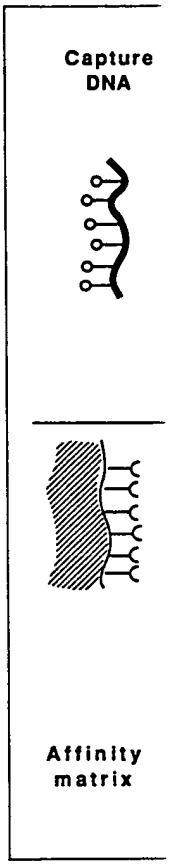

Figure 2–4. Affi
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